TRANSLATION NO. 2402

DATE: 26 Feb. 1969

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SUBMICROSCOPIC STRUCTURE OF PASTEURELLA PESTIS HOLLAND

[Following is the translation of an article by L. N. Kats, Institute of Epidemiology and Microbiology imeni Gamaleya, AMN USSR, Moscow, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology) 43(7): 84-86, 1966. It was submitted on 17 Jun 1965.]

Cytological investigations of P. pestis have been carried out mainly with the help of the light microscope (Lagovaya and Lebedeva, 1931; Pokrovskaya, 1961; Anisimov, 1959, and others). Electron microscope investigations of the plague causative agent are few and have been performed mainly on whole cells (Crocker and Chen, 1956). In the literature known to us only Mishchenko and Kharlampovich (1963) described the structure of this microorganism on ultrathin sections.

We made an electron microscopic study of ultrathin sections of an 18- and 72-hour culture of P. pestis (EV vaccine strain), incubated on blood agar under conditions which were optimum for the formation of capsules. * For the electron microscopic investigation the bacterial cells were fixed with an osmium fixative by the method of Ryter and Kellenberger (1958), dehydrated in alcohols, and included in a mixture of methyl- and butylmethacrylates in a ratio of 1:4. The ultrathin sections were obtained on an LKB-Produkter ultratome with the help of a glass or diamond knife and contrasted with uranyl acetate. Besides this, whole capsular cells were negatively stained with 2% phosphotungstic acid (pH 5.9 and 7.4) without preliminary fixation. The preparations were examined in a JEM-6 C Japanese electron microscope and photographed on MP film with a magnification of 20,000 and 30,000 times.

* The culture was kindly given to us by V. R. Arkhipova, to whom we express our thanks.

On preparations of whole cells, negatively stained with phosphotungstic acid, and on the ultrathin sections the cells of P.pestis were elongated with rounded ends (see Fig. 1 on the inset between pages 128—129). On transverse sections they have an oval or irregular form (Fig. 2 on the inset between pages 128—129).

During negative staining of whole cells the capsule is not apparent. On ultrathin sections the main part of the capsule is disintegrated. Remnants of it can be observed only in some cells in the form of irregularly scattered fibrillar material; the

diameter of individual fibrils was around 30 Å (Fig. 2 and 3 on the inset between pages 128--129). Directly under the fibrillar material of the capsule were membranous structures, surrounding the body of the cell and having on ultrathin sections a sinous configuration. The outer membrane is two-layered (Figure 4 on the inset between pages 128--129) and consists of 2 electron-dense layers with a thickness of 25--30 Å each and an inner layer of the same thickness located between the electron-dense layers. Under the two-layered membrane is a layer of moderate electronoptic density, which in different sectors has a thickness of from 30 to 110 Å (and even up to 1100 Å in the polar sectors of the cell). The outer two-layered membrane and the layer of moderate electron-optic density lying under it, according to the concepts of Claus and Roth (1964), make up the cell wall, which thus in P. pestis has an overall thickness from 120 to 220 Å, and in the polar sectors of some cells sometimes reaches 1200 Å.

Under the cell wall is a two-layered cytoplasmic membrane of the same dimensions and structure as the outer membrane of the cell wall (Fig. 4). Thus, both the outer and the cytoplasmic membrane form alternating layers which are electronoptically more dense, less dense, and again electronoptically dense, i.e., in structure and dimensions are similar to an elementary membrane (Grin, 1964, and others).

However, not the entire surface of the cell has the described typical structure. On several sectors of the surface of the cell it is possible to observe not 2, but 3 two-layered membranes (Fig. 5 on the inset between pages 128--129). In this case it is difficult to judge which of them should be related to the cell wall and which to the cytoplasmic membrane. In P. pestis often there are annular closed membranous structures branching out from the cyto-plasmic membrane inside the cytoplasm. These have been described by a number of authors both for gram-positive and for gram-negative bacteria under the name of mesosome (Iterson and Leene, 1964a, b; Biryuzova and Meysel', 1964, and others). The cytoplasm in P. pestis, just as in other bacteria, is filled with a granular component. Individual osmiophylic granules have a diameter of 150--200 Å, which corresponds to the dimensions of bacterial ribo-somes (Fig. 4). In P. pestis the ribosomes are uniformly distrib-uted along the periphery of the cell, but sometimes form an accumu-lation at the poles of the cell (Fig. 1, above). In addition to the ribosomes, also revealed in the cytoplasm are electronoptic inclusions which have a fine granular structure and often are surrounded by a corolla of ribosomes (Fig. on the inset between pages 128--129). The nature of these formations is still unclear.

Nucleoid occupies a considerable, usually central, portion of the cell and represents osmiophylic material with irregularly scat-tered thin osmiophylic fibrils. The fibrils have a diameter of 20--25 Å, which corresponds to the dimensions of fibrils of bacter-ial DNA (Fig. 1 and 2). Often the central portion of the nucleoid

also contains electronoptic granular material, apparently representing sectors of the cytoplasm in the nucleoid zone.

Thus, P. pestis has a structure which is similar to the structure of previously described gram-negative pathogenic and saprophytic bacteria. Just as with other gram-negative bacteria, in P. pestis a three-layered cytoplasmic membrane is revealed [based on the data of Mishchenko and Kharlampovich (1963) in this microorganism the membrane is single-layered]. The cytoplasmic membrane forms invaginates of the mesosome type within the cytoplasm (based on data of Mishchenko and Kharlampovich, mesosomes are lacking in P. pestis).

Conclusions

1. Electron microscopic investigations of capsular forms of P. pestis (vaccine strain) were carried out both on whole capsular cells, negatively stained with phosphotungstic acid, and on ultrathin sections.

2. On ultrathin sections the capsule is represented in the form of scraps of fibrillar material. Under the capsule there are 2 two-layered membranes: an outer membrane of the cell wall and a cytoplasmic membrane, separated by a layer of moderate electronoptic density.

3. In the cytoplasm ribosomes are revealed, as well as membranous structures of the mesosome type, unindentified electrondense inclusions and nucleoids.

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